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(54) PREPARATION OF CHIMAERIC ANTIBODIES USING THE RECOMBINANT PCR STRATEGY

HERSTELLUNG SCHIMÄRER ANTIKÖRPER DURCH DIE REKOMBINANTE PCR-STRATEGIE

PREPARATION D'ANTICORPS CHIMERIQUES PAR LA TECHNIQUE DE REACTION EN CHAINE DE POLYMERASE RECOMBINANTE

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EP 0 552 266 B1

Description

[0001] The present invention relates to the preparation of chimaeric antibodies. The invention is typically applicable to the production of humanised antibodies.

[0002] Antibodies typically comprise two heavy chains linked together by disulphide bonds and two light chains. Each light chain is linked to a respective heavy chain by disulphide bonds. Each heavy chain has at one end a variable domain followed by a number of constant domains. Each light chain has a variable domain at one end and a constant domain at its other end. The light chain variable domain is aligned with the variable domain of the heavy chain. The light chain constant domain is aligned with the first constant domain of the heavy chain. The constant domains in the light and heavy chains are not involved directly in binding the antibody to antigen.

[0003] The variable domains of each pair of light and heavy chains form the antigen binding site. The domains on the light and heavy chains have the same general structure and each domain comprises a framework of four regions, whose sequences are relatively conserved, connected by three complementarily determining regions (CDRs). The four framework regions largely adopt a beta-sheet conformation and the CDRs form loops connecting, and in some cases forming part of, the beta-sheet structure. The CDRs are held in close proximity by the framework regions and, with the CDRs from the other domain, contribute to the formation of the antigen binding site. CDRs and framework regions of antibodies may be determined by reference to Kabat et al ("Sequences of proteins of immunological interest" US Dept. of Health and Human Services, US Government Printing Office, 1987).

[0004] The preparation of an altered antibody in which the CDRs are derived from a different species to the variable domain framework regions is disclosed in EP-A-0239400. The CDRs may be derived from a rat or mouse monoclonal antibody. The framework of the variable domains, and the constant domains, of the altered antibody may be derived from a human antibody. Such a humanised antibody elicits a negligible immune response when administered to a human compared to the immune response mounted by a human against a rat or mouse antibody. Humanised CAM-PATH-I antibody is disclosed in EP-A-0328404.

[0005] The technique of "overlap extension" involves the use of oligonucleotide primers complementary to a template nucleotide sequence and the polymerase chain reaction (PCR) to generate DNA fragments having overlapping ends. These fragments are combined in a "fusion" reaction in which the overlapping ends anneal allowing the 3' overlap of each strand to serve as a primer for the 3' extension of the complementary strand. Ho et al (Gene, 77, 51-59 (1989)) describe the use of this technique to introduce specific alterations in a nucleotide sequence by incorporating nucleotide changes into the overlapping oligo primers. Using this technique of site-directed mutagenesis, those variants of the mouse major histocompatibility complex class-I gene were generated cloned and analysed.

[0006] Horton et al (Gene, 77 61-68 (1999)) describe a technique of gene splicing by overlap extension (SOE). The technique allows the production of a hybrid length of DNA, AD, by splicing two pieces of DNA, AB and CD, which are produced by a PCR using primers A, B, C and D. At least part of the primers B and C are complementary to each other. The fragments AB and CD produced by PCR are mixed to allow the positive strand of AB to anneal to the negative strand of CD. The overlap between B and C allows the two strands to prime extension of each other. Primers A and D are used to prime a PCR reaction of the extended strands.

[0007] The above technique was used to splice a portion (CD) of the mouse H-2K^b gene between upstream and downstream regions (AB and EF respectively) of the corresponding upstream and downstream parts of the H-2L^d gene. All three fragments, AB, CD and EF were produced by PCR, using primers A to F. The three fragments were joined by two rounds of SOE, the first one producing a fragment AD (ie. AB-CD) and the second producing the product AF (ie. AB-CD-EF).

[0008] According to the present invention, a method has now been devised of producing a chimaeric antibody in which the CDR of a first antibody is spliced between the framework regions of a second antibody.

[0009] In general, the technique of the present invention is performed using a template comprising two framework regions, AB and CD, and between them, the CDR which is to be replaced by a donor CDR. Primers A and B are used to amplify the framework region AB, and primers C and D used to amplify the framework region CD. However, the primers B and C also contain, at their 5' ends, additional sequence corresponding to all or at least part of the donor CDR sequence. Primers B and C overlap by a length sufficient to permit annealing of their 5' ends to each other under conditions which allow a polymerase chain reaction (PCR) to be performed and thereby incorporate all of the donor CDR sequence. The amplified regions AB and CD may undergo SOE to produce the chimaeric product in a single reaction.

[0010] According to one aspect the present invention provides a method for producing a double- or single-stranded DNA of formula

5' F1-M-F2 3'

encoding an antibody chain or fragment thereof in which at least one of the complementarity determining regions (CDRs) of the variable region of the antibody chain is derived from a first mammalian antibody, and the framework of

the variable region is derived from a second, different mammalian antibody, wherein M comprises DNA encoding a CDR of the first antibody and F1 and F2 encode sequences flanking M, which method comprises;

(i) preparing a single- or double-stranded DNA template of the formula

5' f1-H-f2 3'

wherein H comprises DNA encoding a CDR of a different specificity from M and f1 and f2 are substantially homologous to F1 and F2 respectively (i.e. f1 and f2 may have minor changes compared to F1 and F2 respectively)

(ii) obtaining DNA oligonucleotide primers A, B, C and D wherein

A

- comprises a sequence a^1 which has a 5' end corresponding to the 5' end of F1 and which is identical to a corresponding length (i.e. a sequence of the same number of nucleotides and with the identical or complementary sequence) of the sequence F1,
- is oriented in a 5' to 3' direction towards H;

B consists of the sequence

5' b^1-b^2 3'

wherein

- b^1 comprises a sequence complementary to a corresponding length of M and has a 3' end which is complementary to the 5' end of M, and
- b^2 is complementary to a sequence of corresponding length in F1 and has a 5' end which starts at the nucleotide complementary to the 3' end of F1;

C consists of the sequence

5' c^1-c^2 3'

wherein

- c^1 comprises a sequence identical to the corresponding length of M and has a 3' end which corresponds to the 3' end of M, and
- c^2 is identical to a sequence of corresponding length in F2 and has a 5' end which starts at the nucleotide corresponding to the 5' end of F2;

D

- comprises a sequence d^1 which has a 5' end complementary to the 3' end of F2 and which is complementary to a corresponding length of F2, and
- is oriented in a 5' to 3' direction towards H;

and wherein b^1 and c^1 overlap by a length sufficient to permit annealing of their 5' ends to each other under conditions which allow a PCR to be performed;

(iii) performing, in any desired order, PCR reactions with primer pairs A,B and C,D on the template prepared in (i) above; and

(iv) mixing the products obtained in (iii) above and performing a PCR reaction using primers A and D.

[0011] The oligonucleotides may be of any convenient size.

[0012] Preferably F1 and F2 each encode at least one human antibody framework region and optionally further CDRs.

Preferably H encodes a CDR of said human antibody. Preferably M encodes a non-human CDR region, most preferably a murine or rodent CDR.

[0013] Primers A and D will usually be at least 12, for example at least 15 nucleotides, and more usually from 20 to 30 nucleotides in length. If desired primers A and D may contain at least one restriction endonuclease recognition site within nucleotides of their 5' ends. Primers B and C will usually be at least 20, for example at least 30 nucleotides in length. More usually, these primers will be over 40, for example 45 to 60 nucleotides long. It is generally possible to synthesise oligonucleotides of up to 200 nucleotides in length. Generally primers A, B, C and D will thus each be from 15 to 200 nucleotides in length.

[0014] The length of overlap between b^1 and c^1 may depend on a number of factors, including the total length of B and C and the particular base composition of the region of the overlap. However, the overlap will usually be at least 12, for example at least 15, nucleotides. According to one embodiment, the sequences b^1 and c^1 within the primers B and C are the same number of nucleotides in length. In a preferred embodiment of the invention b^1 and c^1 are both the length of M and thus the overlap is also this length.

[0015] Usually, the distance between the 3' end of primer A and the 5' end of H will be at least 15 nucleotides. More usually, the distance will be the length of f1 minus the length of A itself. Similarly, the distance between the 3' end of D and the region H will also be at least 15 nucleotides, and more usually the length of f2 minus the length of D itself. According to one embodiment the sequences a^1, b^2, c^2 and d^1 of primers A, B, C and D respectively are each from 15 to 30 nucleotides in length.

[0016] It will be appreciated that the entire sequence of M and the 5' and 3' regions of F1 and F2 will be determined by the sequence of the primers A, B, C and D.

[0017] It is therefore considered inappropriate in this situation to refer to "homology" between these primers and any parts of the sequence F1, M or F2. Instead, the term "corresponding length" as used herein means a sequence of the same number of nucleotides and with the identical (or complementary) sequence.

[0018] With reference to step (i) above, the sequences f1 and f2 will be substantially homologous to F1 and F2 respectively in that the primers A to D may be used to introduce minor changes to f1 and f2 in the regions of these primer sequences.

[0019] The regions F1 and F2 comprise DNA encoding at least part of the framework regions either side of the CDR M. F1 and F2 may also encode regions flanking these sequences, for example into and beyond DNA encoding further CDRs.

[0020] According to another aspect, the present invention provides an oligonucleotide 30 to 110 nucleotides in length which consists of the sequence:

5' o^1-o^2 3'

wherein o^1 comprises at least 15 nucleotides of a sequence of a CDR region of non-human origin and o^2 comprises at least 15 nucleotides of a framework region of human origin. This oligonucleotide is suitable for use as a primer in the process described above.

[0021] According to a still further aspect, the present invention provides a method for producing a double- or single-stranded DNA of formula

5' F1-M1-F2-M2-F3-M3-F4 3'

encoding an antibody chain or fragment thereof in which the three complementarity determining regions (CDRs) of the variable region of the antibody chain are derived from a first mammalian antibody, and the four framework regions of the variable domain are derived from a second, different mammalian antibody, wherein M1, M2 and M3 comprise DNA encoding CDRs of the first antibody and F1, F2, F3 and F4 comprise framework sequences flanking the CDRs M1, M2 and M3, which method comprises;

(i) preparing a single- or double-stranded DNA template of the formula

5' f1-H1-f2-H2-f3-H3-f4 3'

wherein H1, H2 and H3 comprises DNA encoding CDRs of a different specificity from M1, M2 and M3, and f1, f2, f3 and f4 are substantially homologous to F1, F2, F3 and F4 respectively (i.e. f1 and f2 may have minor changes compared to F1 and F2 respectively)

(ii) obtaining DNA oligonucleotide primers A, B, C, D, E, F, G and H wherein

A

- comprises a sequence a^1 which has a 5' end corresponding to the 5' end of F1 and which is identical to a corresponding length (i.e. a sequence of the same number of nucleotides and with the identical or complementary sequence) of the sequence F1,
- is oriented in a 5' to 3' direction towards H1;

B consists of the sequence

5' b^1-b^2 3'

wherein

- b^1 comprises a sequence complementary to a corresponding length of M1 and has a 3' end which is complementary to the 5' end of M1, and
- b2 is complementary to a sequence of corresponding length in F1 and has a 5' end which starts at the nucleotide complementary to the 3' end of F1;

C consists of the sequence

5' c^1-c^2 3'

wherein

- c^1 comprises a sequence identical to the corresponding length of M1 and has a 3' end which corresponds to the 3' end of M1, and
- c^2 is identical to a sequence of corresponding length in F2 and has a 5' end which starts at the nucleotide corresponding to the 5' end F2;

D consists of the sequence

5' d^1-d^2 3'

wherein

- d^1 comprises a sequence complementary to a corresponding length of M2 and has a 3' end which is complementary to the 5' end of M2, and
- d^2 is complementary to a sequence of corresponding length in F2 and has a 5' end which starts at the nucleotide complementary to the 3' end of F2;

E consists of the sequence

5' e^1-e^2 3'

wherein

- e^1 comprises a sequence identical to the corresponding length of M2 and has a 3' end which corresponds to the 3' end of M2, and
- e^2 is identical to a sequence of corresponding length in F3 and has a 5' end which starts at the nucleotide corresponding to the 5' end F3;

F consists of the sequence

5' f^1-f^2 3'

wherein

- f^1 comprises a sequence complementary to a corresponding length of M3 and has a 3' end which is com-

plementary to the 5 ' end of M3, and

- f^2 is complementary to a sequence of corresponding length in F3 and has a 5' end which starts at the nucleotide complementary to the 3' end of F3;

G consists of the sequence

5' g^1-g^2 3'

wherein

- g^1 comprises a sequence identical to the corresponding length of M3 and has a 3' end which corresponds to the 3' end of M3, and
- g^2 is identical to a sequence of corresponding length in F4 and has a 5' end which starts at the nucleotide corresponding to the 5' end F4;

H

- comprises a sequence h^1 which has a 5' end complementary to the 3' end of F4 and which is complementary to a corresponding length of F4, and
- is oriented in a 5' to 3' direction towards H3;

and wherein the pairs b^1 and c^1, d^1 and e^1, and f^1 and g^1 overlap by a length sufficient to permit annealing of their 5' ends to each other under conditions which allow a PCR to be performed;

(iii) performing, in any desired order, PCR reactions with primer pairs A,B; C,D; E,F and G,H on the template prepared in (i) above to obtain DNA fragments AB, CD, EF and GH; and

(iv) splicing the fragments obtained in (iii) above to obtain the desired DNA.

[0022] According to one embodiment, F4 comprises the framework sequence flanking the CDR M3 and DNA encoding all or part of the constant region of the antibody chain.

Step (iv) may be performed by:

(iva) mixing fragments AB and CD with primers A and D and performing a PCR to obtain a DNA fragment AD;
(ivb) mixing, before, during or following step (iva) above, fragments EF and GH with primers E and H and performing a PCR to obtain a DNA fragment EH; and
(ivc) mixing fragments AD and EH with primers A and H to obtain the desired DNA.

Alternatively step (iv) may be performed by:

(iva) mixing fragments CD and EF with primers C and F and performing a PCR to obtain a DNA fragment CF; and EITHER:
(ivb-1) mixing fragments AB and CF with primers A and F and performing a PCR to obtain a DNA fragment AF; and
(ivc-1) mixing fragments AF and GH with primers A and H to obtain the desired DNA; OR:
(ivb-2) mixing fragments CF and GH with primers C and H and performing a PCR to obtain a DNA fragment CH; and
(ivc-2) mixing fragments AB and CH with primers A and H to obtain the desired DNA.

Description of the drawings

[0023]

Figure 1 illustrates a process according to the present invention. The dark box indicates DNA sequence from a murine CDR region which is inserted between framework regions of the CAMPATH antibody, replacing the original CDR (unshaded box). A, B, C and D indicate the PCR primers used, with half-arrows indicating their 5' to 3' orientation.

Figure 2 shows in detail the key sequences involved in the process illustrated in Figure 1.

Figure 3 is a schematic illustration of how the process of the invention may be used to replace all three CDR regions of an antibody.

Figure 4 illustrates in further detail one configuration of primers which may be used in the present invention.

[0024] Possible variations in the F1 and F2 DNA regions are apparent by contrasting the embodiments of the invention illustrated in Figures 1 and 3.

[0025] In Figure 1, a process according to the invention is illustrated showing the replacement of a single CDR DNA. The region F2 in Figure 1 is between primers "C" and "D", starting at the 5' end of c^2 as defined above to the complement of the 5' end of "D". This region encodes a total of 3 framework regions, 2 CDRs and the whole heavy chain constant region incorporating a stop codon within primer D. In contrast, the DNA of F1, 5' to the CDR being replaced, contains a single framework and no CDRs.

[0026] In Figure 3, the DNA between primers "C" and "D" encodes a single framework region. This is because the process illustrated shows the replacement of all 3 CDRs of DNA encoding the variable region of an antibody. With this arrangement, it should be noted that primer "D" comprises not only the sequence of d^1 but also additional 5' sequence encoding part of a second CDR region.

[0027] When the DNA encoding all 3 CDRs of an antibody chain is to be replaced, the arrangement of Figure 3 may be used.

[0028] Thus, a first set of 4 primers, "A", "B", "C" and "D" (as defined above for A, B, C and D) are used to replace all of a first CDR (CDR1) and at least part of a second CDR, (CDR2). A second set of primers, "E", "F", "G" and "H" (defined as for A, B, C and D respectively) are used to replace a third CDR (CDR3) and at least part of CDR2. In order to ensure the replacement of CDR2, primers "D" and "E" must overlap by a length sufficient to permit annealing of their 5' ends to each other under conditions which allow a PCR to be performed. In essence, replacement of CDR2 is accomplished by a set of four primers, "C", "D", "E" and "F", defined as for A, B, C and D respectively.

[0029] In the embodiment of the invention illustrated by Figure 3, fragments AB and CD are annealed to provide fragment AD, and fragments EF and GH are spliced to provide fragment EH. Finally AD is spliced with EH to provide fragment AH, encoding a variable region in which all 3 CDRs are replaced.

[0030] Other arrangements by which all 3 CDR DNAs may be replaced in a DNA encoding a variable region using primers "A" to "H" as illustrated in Figure 3 include performing reactions with primer pairs "A" + "B", "C" + "D", "E" + "F" and "G" + "H" as illustrated in Figure 3(1), splicing fragments CD and EF together to produce a fragment CF, and splicing this fragment with either first fragment AB and then GH, or vice versa.

[0031] Alternatively, the DNA encoding the 3 CDRs may be replaced sequentially. A first reaction using primers "A", "B", "C" and "H" (as shown in Figure 3 and defined as for primers A to D) may be used to replace CDR1, in accordance with the present invention. A second set of reactions, using primers "A", "E", "D" and "H" (as shown in Figure 3 and defined as for primers A to D) replaces CDR2. A final set of reactions, using primers "A", "F", "G" and "H" replaces CDR3.

[0032] The primers A and D may also, at their 5' ends contain additional sequences which represent, for example, restriction endonuclease recognition sequences not represented in f1 or f2.

[0033] The sequences of A and D 5' to a^1 and d^1 will be ignored when considering the degree of homology between f1 and F1, and f2 and F2. Similarly, if F1 and/or F2 are shorter than f1 and/or f2 respectively, the additional sequences of f1/f2 for which F1/F2 have no counterpart will also be ignored when measuring the degree of homology.

[0034] All the primers may contain a number, for example 1 to 10, such as 2 to 5 nucleotide mismatches between the f1/f2 sequences and the corresponding or complementary primer sequences. These mismatches may be used to design desired coding changes in the sequences of F1 and F2 when compared with f1 and f2.

[0035] The process of the invention may be used to produce a chimaeric antibody or fragment thereof in which any one of the CDR regions are replaced. It may also be used to replace any two, or all three CDR regions of an antibody variable region.

[0036] The process of the invention may be used to replace the DNA encoding one or more CDRs of a complete antibody light or heavy chains. Fragments of DNA encoding at least one CDR region may be used. For example, it is possible to produce antibody fragments such as Fab, $F(ab)_2$ or Fv fragments, in which the DNA encoding one or both of the light or heavy chains has been subjected to the process of the invention.

[0037] DNA encoding framework regions and CDRs of antibodies will often be present in a vector, for example an expression vector. In some cases, it will be necessary or desirable that one or both of the primers A and D (or at least their regions a^1 and d^1) correspond to vector sequences, rather than sequences of one of the framework regions flanking the CDR being replaced.

[0038] The DNA produced according to the invention may be cloned into any suitable replication or expression vector

and introduced into a bacterial, yeast, insect or mammalian cell to produce chimaeric antibody. Examples of suitable systems for expression are described below.

[0039] The antibody chain may be co-expressed with a complementary antibody chain. At least the framework of the variable region and the or each constant region of the complementary chain generally are derived from the said second species also. A light chain and a heavy chain may be co-expressed. Either or both-chains may have been prepared by the process of the invention. Preferably the CDRs of both chains are derived from the same selected antibody. An antibody comprising both expressed chains can be recovered.

[0040] The antibody preferably has the structure of a natural antibody or a fragment thereof. The antibody may therefore comprise a complete antibody, a $(Fab')_2$ fragment, a Fab fragment, a light chain dimer or a heavy chain. The antibody may be an IgG, such as an IgG1, IgG2, IgG3 or IgG4 IgM, IgA, IgE or IgD. Alternatively, the antibody may be a chimaeric antibody of the type described in WO 86/01533.

[0041] A chimaeric antibody according to Wo 86/01533 comprises an antigen binding region and a non-immunoglobulin region. The antigen binding region is an antibody light chain variable region or heavy chain variable region. Typically, the chimaeric antibody comprises both light and heavy chain variable regions. The non-immunoglobulin region is fused at its C-terminus to the antigen binding region. The non-immunoglobulin region is typically a non-immunoglobulin protein and may be an enzyme region, a region derived from a protein having known binding specificity, from a protein toxin or indeed from any protein expressed by a gene. The two regions of the chimaeric antibody may be connected via a cleavable linker sequence.

[0042] The invention is preferably employed to humanise an antibody, typically a monoclonal antibody and, for example, a rat or mouse antibody. The framework and constant regions of the resulting antibody are therefore human framework and constant regions whilst the CDRs of the light and/or heavy chain of the antibody are rat or mouse CDRs. Preferably all CDRs are rat or mouse CDRs. The antibody produced in accordance with the present invention may be a human IgG such as IgG1, IgG2, IgG3, IgG4; IgM; IgA; IgE or IgD carrying rat or mouse CDRs.

[0043] The process of the invention is carried out in such a way that the resulting chimaeric antibody retains the antigen binding capability of the non-human antibody from which the CDR region(s) is/are derived.

[0044] The starting antibody is typically an antibody of a selected specificity. In order to ensure that this specificity is retained, the variable region framework of the antibody is preferably the closest variable region framework of an antibody of another species. By "about the closest" is meant about the most homologous in terms of amino acid sequences. Preferably there is a homology of at least 50% between the two variable regions.

[0045] There are four general steps to produce a humanised antibody by the method according to the invention. These are:

(1) determining the nucleotide and predicted amino acid sequence of the starting antibody light and heavy chain variable regions;
(2) designing the chimaeric antibody, i.e. deciding which antibody framework region to use during the process;
(3) identifying the oligonucleotides A, B, C, and D and use of these primers in a series of PCR reactions to produce DNA encoding the humanised antibody; and
(4) the transfection of a suitable host cell line with the DNA and expression of the humanised antibody.

[0046] These four steps are explained below in the context of humanising an antibody. However, they may equally well be applied when reshaping to an antibody of a non-human species.

Step 1: Determining the nucleotide and predicted amino acid sequence of the antibody light and heavy chain variable regions

[0047] To make a chimaeric antibody only the amino acid sequence of antibody's heavy and light chain variable regions needs to be known. The sequence of the constant regions is irrelevant because these do not contribute to the humanising strategy. The simplest method of determining the variable region amino acid sequence of an antibody is from cloned cDNA encoding the heavy and light chain variable region.

[0048] There are two general methods for cloning heavy and light chain variable region cDNAs of a given antibody; (1) via a conventional cDNA library, or (2) via PCR. Both of these methods are widely known. Given the nucleotide sequence of the cDNAs, it is a simple matter to translate this information into the predicted amino acid sequence of the antibody variable regions.

Step 2: Designing the chimaeric antibody

[0049] There are several factors to consider in deciding which human antibody sequence to use during the humanisation. The humanisation of light and heavy chains are considered independently of one another, but the reasoning is

basically similar for each.

[0050] This selection process is based on the following rationale: A given antibody's antigen specificity and affinity is primarily determined by the amino acid sequence of the variable region CDRs. Variable region framework residues have little or no direct contribution. The primary function of the framework regions is to hold the CDRs in their proper spacial orientation to recognize antigen. Thus the substitution of rodent CDRs into a human variable region framework is most likely to result in retention of their correct spacial orientation if the human variable region is highly homologous to the rodent variable region from which they originated. A human variable region should preferably be chosen therefore that is highly homologous to the rodent variable region(s).

[0051] A suitable human antibody variable region sequence can be selected as follows:

1. Using a computer program, search all available protein (and DNA) databases for those human antibody variable region sequences that are most homologous to the rodent antibody variable regions. The output of a suitable program is a list of sequences most homologous to the rodent antibody, the percent homology to each sequence, and an alignment of each sequence to the rodent sequence. This is done independently for both the heavy and light chain variable region sequences. The above analyses are more easily accomplished if only human immunoglobulin sequences are included.

2. List the human antibody variable region sequences and compare for homology. Primarily the comparison is performed on length of CDRs, except CDR3 of the heavy chain which is quite variable. Human heavy chains and Kappa and Lambda light chains are divided into subgroups; Heavy chain 3 subgroups, Kappa chain 4 subgroups, Lambda chain 6 subgroups. The CDR sizes within each subgroup are similar but vary between subgroups. It is usually possible to match a rodent Ab CDR to one of the human subgroups as a first approximation of homology. Antibodies bearing CDRs of similar length are then compared for amino acid sequence homology, especially within the CDRs, but also in the surrounding framework regions. The human variable region which contains the most homologous CDRs is chosen as the framework for humanisation.

Step 3: Identification and use of the oligonucleotides A, B, C and D

[0052] The general principles for designing primers for PCR are well known, eg. as described by R.K. Saiki ("The Design and Optimisation of the PCR" in "PCR Technology", Ed H.A. Erlich, Stockton Press, (1989)). In addition, specific factors can be considered for each CDR replacement. Where necessary, or desired, the 5' ends of A and/or D may encode part or all of a second and/or third CDR. The primers, A and D, may also include at their 5' ends restriction enzyme sites. These sites can be designed according to the vector which will be used to clone the humanised antibody from the final PCR reaction. The primers B and C must be long enough to overlap by at least a length sufficient to permit annealing of their 5' ends to each other under conditions which allow a PCR to be performed. This will usually require an overlap of at least 12, and preferably at least 15 nucleotides. One or more of the four primers may differ from their template sequences by one or more nucleotides. These differences may be used to introduce desired coding changes into the framework regions of the antibody.

[0053] The primers are then used in a series of PCR reactions using the appropriate template to generate the DNA encoding the humanised antibody. PCR reactions may be carried out as described by Saiki et al, Science, 239, 487-491 (1988). At each stage the desired product of the PCR reaction may be purified as necessary, for example using selective filtration and if necessary the identity of the product can be established, for example by gel electrophoresis.

Step 4: Transfection and expression of the reshaped antibody

[0054] Following the reactions to produce the DNA encoding the chimaeric antibody, the DNAs are linked to the appropriate DNA encoding light or heavy chain constant region, cloned into an expression vector, and transfected into a suitable host cell line, preferably a mammalian cell line. These steps can be carried out in routine fashion. A chimaeric antibody may therefore be prepared by a process comprising:

a) preparing a first replicable expression vector including a suitable promoter operably linked to a DNA sequence which encodes at least a variable region of an Ig heavy or light chain, the variable region comprising framework regions from a first antibody and CDRs from a second antibody of different specificity;
b) if necessary, preparing a second replicable expression vector including a suitable promoter operably linked to a DNA sequence which encodes at least the variable region of a complementary Ig light or heavy chain respectively;
c) transforming a cell line with the first or both prepared vectors; and
d) culturing said transformed cell line to produce said altered antibody.

[0055] Preferably the DNA sequence in step a) encodes both the variable region and the or each constant region of the antibody chain. The antibody can be recovered and purified. The cell line which is transformed to produce the altered antibody may be a Chinese Hamster ovary (CHO) cell line or an immortalised mammalian cell line, which is advantageously of lymphoid origin, such as a myeloma, hybridoma, trioma or quadroma cell line. The cell line may also comprise a normal lymphoid cell, such as a B-cell, which has been immortalised by transformation with a virus, such as the Epstein-Barr virus. Most preferably, the immortalised cell line is a myeloma cell line or a derivative thereof.

[0056] Although the cell line used to produce the chimaeric antibody is preferably a mammalian cell line, any other suitable cell line, such as a bacterial cell line or a yeast cell line, may alternatively be used. In particular, it is envisaged that E. coli - derived bacterial strains could be used.

[0057] It is known that some immortalised lymphoid cell lines, such as myeloma cell lines, in their normal state secrete isolated Ig light or heavy chains. If such a cell line is transformed with the vector prepared in step (a) it will not be necessary to carry out step (b) of the process, provided that the normally secreted chain is complementary to the variable region of the Ig chain encoded by the vector prepared in step (a).

[0058] However, where the immortalised cell line does not secrete or does not secrete a complementary chain, it will be necessary to carry out step (b). This step may be carried out by further manipulating the vector produced in step (a) so that this vector encodes not only the variable region of a chimaeric antibody light or heavy chain, but also the complementary variable region.

[0059] Alternatively, step (b) is carried out by preparing a second vector which is used to transform the immortalised cell line. This alternative leads to easier construct preparation, but may not be as preferred as the first alternative in that production of antibody may be less efficient.

[0060] In the case where the immortalised cell line secretes a complementary light or heavy chain, the transformed cell line may be produced for example by transforming a suitable bacterial cell with the vector and then fusing the bacterial cell with the immortalised cell line by spheroplast fusion. Alternatively, the DNA may be directly introduced into the immortalised cell line by electroporation or other suitable method.

[0061] An antibody is consequently produced in which CDRs of a variable region of an antibody chain are homologous with the corresponding CDRs of an antibody of a first mammalian species and in which the framework of the variable region and the constant regions of the antibody are homologous with the corresponding framework and constant regions of an antibody of a second, different, mammalian species. Typically, all three CDRs of the variable region of a light or heavy chain are derived from the first species.

[0062] The antibody may be an IgG, such as IgG1, IgG2, IgG3 or IgG4 IgM, IgA, IgE or IgD. Alternatively, the antibody may be a chimaeric antibody of the type described in WO 86/01523.

[0063] The recombinant PCR technique of the present invention should allow the generation of fully humanised MAb DNA sequences in only two days using three rounds of PCR reactions (Fig. 3). Site-directed mutagenesis (Jones et al., Nature, 321, 522-525 (1986); Riechmann et al., Nature, 332, 323-327 (1988)) and oligonucleotide gene synthesis (Queen et al., Proc. Natl. Acad. Sci. U.S.A., 86, 10029-10033 (1989)) have previously been used for the humanisation of antibodies. The above method has benefits over these techniques in that smaller oligonucleotides are required in the procedure, even to transfer large CDRs such as the 19 amino acid CDRH2 present in a number of human IgG subgroup III heavy chains (Cleary et al., Cell, 44, 97-106 (1986)). For example, as illustrated in Figure 4, where the primary PCR products are designed to overlap in the middle of the CDR by 15 bp, the transfer of a 57 bp CDR onto the appropriate FR requires oligonucleotides of a maximum of 51 bp, assuming a homology of 15 bp corresponding to the FR target sequence (Higuchi, Using PCR to engineer DNA, in "PCR Technology" Ed. H.A. Erlich, Stockton Press (1989)).

[0064] The technique of the invention is also advantageous over site-directed mutagenesis in that all operations can be performed upon ds DNA without the need for subcloning between ds and ss vectors, thus decreasing the time and effort required to generate the humanised product.

[0065] The invention is illustrated by the following example.

EXAMPLE 1

(a) Recombinant PCR grafting of DX48 CDRH1 onto a human background

[0066] The objective was to graft a heavy chain CDR1 (CDRH1) from a rat anti-digoxin mAb (DX48) onto a human Ig backbone. The template used for the recombinant PCR was the previously humanised CAMPATH-1H heavy chain (Riechmann et al., Nature, 332, 323-327 (1988)), a human IgGI heavy chain with NEW (Saul et al., J. Biol. Chem., 253, 585-597 (1978)) V region, which had been re-engineered from genomic into cDNA configuration, and had subsequently undergone site-directed mutagenesis to replace CAMPATH-1H CDRH2 and CDRH3 sequences with rat DX48 CDRH2 and CDRH3 yielding HUMDXCH.23 ss template in M13 (SEQ ID NO: 1).

[0067] PCR reactions (Saiki et al., Science, 239, 487-491 (1988)) were carried out using ss HUMDXCH.23 template prepared by the method of Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Edn., Cold Spring Harbor

Laboratory (1989). The reactions were performed in a programmable heating block (Hybaid) using 25 rounds of temperature cycling (94°C for 1 min, 50°C for 2 min, and 72°C for 3 min) followed by a final 10 min step at 72°C. 1 μg of each primer, 50 ng of template and 2.5 Units of Tag polymerase (Perkin Elmer Cetus) were used in a final volume of 100 μl with the reaction buffer as recommended by the manufacturer. Synthetic oligonucleotides were made on a 7500 DNA Synthesizer (Milligen).

[0068] The approach used is summarised in Fig. 1. Primers used:

A : SEQ ID NO: 2:
B : SEQ ID NO: 3:
C : SEQ ID NO: 4:
D : SEQ ID NO: 5:

Two PCR reactions were carried out using the primer pairs A and B, and C with D respectively. Primers A and D correspond to positive and negative strand oligonucleotides incorporating the HindIII sites at the 5' and 3' termini of the HUMDXCH.23 insert. Figure 2 shows the nucleotide sequence of three regions of the HUMDXCH.23 insert incorporating; the first 42 bp at the 5' end of the insert including the start codon of the CAMPATH-1H leader sequence; the 3' 27 bp of FRH1, the whole length of CDRH1 and the 5' 27 bp of FRH2 from CAMPATH-1H; and the final 27 bp at the 3' terminus of the insert including the stop codon at the end of CAMPATH-1H constant region (CH3). The sequences are separated by 117 bp and 1206 bp respectively. Primer B possesses negative strand sequence from the 3' end of the CAMPATH-1H FRH1 region (with point mutations to convert Phe 27 and Thr 30 of CAMPATH-1H back to the Ser residues present in the NEW FRH1) together with CDRH1 sequence of DX48 in place of the CAMPATH-1H CDRH1 (Fig. 2). Primer C is made up of the positive strand sequence of DX48 CDRHI, complementary to the CDRH1 region of primer B, running into the 5' end of the Campath-1H FRH2 (Fig. 2). In the first round of the AB and CD PCR reactions the HUMDXCH.23 negative strand is synthesised from primers B and D respectively (Fig. 1). In subsequent cycles fragments AB and CD (SEQ ID NO: 6 AND NO: 7 respectively) are amplified (Figs. 1 and 2). The products of the two reactions thus constitute the whole length of the HUMDXCH.23 insert but with the point mutations stated above and the Campath-1H CDRH1 replaced by the CDRH1 sequence of DX48. Fragments AB and CD both possess the DX48 CDRH1 sequence such that on denaturation and reannealing the overlapping sequences can anneal.

[0069] Excess primers were removed from the AB and CD PCR reactions by selective filtration on a Centricon 100 (Higuchi et al., Nucl. Acids Res., 16, 7351-7367 (1988); Amicon). 50 μl of each reaction was placed into 2 ml of TE (10mM Tris-HCl pH 8, 0.1 mM EDTA) and mixed in the upper reservoir of the Centricon 100. The manufacturer's protocol was followed using a 25 min centrifugation in a fixed-angle rotor at 1000 x G, and the PCR products recovered in a 40 μl retentate.

[0070] 10 μl of the Centricon 100 retentate was subjected to a recombinant PCR reaction with primers A and D (Fig. 1) using the same conditions as performed in the primary PCR reactions above. The positive strand of fragment AB and the negative strand of CD contain the complementary DX48 CDRH1 sequences at their 3' ends, and in the first PCR cycle can anneal and serve as primers for one another. Extension of the overlap produces the recombinant product fragment AD containing the transplanted DX48 CDRH1, and this is amplified by primers A and D in the subsequent rounds of PCR (Figs. 1 and 2). The remaining strands of fragments AB and CD, which are complementary at their 5' ends, are not able to prime each other, but can act as templates for primers A and D. These generate more of the primary PCR products, although these fragments are not amplified in an exponential manner due to the absence of primers B and C in the reaction.

[0071] Gel-purified PCR products were analysed on an agarose gel containing 0.8% Type II: Medium EEO Agarose (Sigma) in 89 mM Tris-borate/2 mM EDTA, and visualised by staining with ethidium bromide. The expected sizes of the fragments were as follows: AB, 207 bp; CD, 1285 bp; AD, 1471 bp. The predominant band observed in each case was of the expected size, although additional minor bands also appeared in reaction AD.

(b) Cloning and sequencing of the recombinant PCR product

[0072] Fragment AD (SEQ ID NO: 8) was gel eluted, digested with HindIII (BRL) and cloned into the HindIII site of pUC-18 (BRL). The nucleotide sequence of a clone containing the recombinant molecule was determined by plasmid priming following the dideoxy chain-termination method (Sanger et al., Proc. Natl. Acad. Sci. U.S.A., 74, 5463-5467 (1977)) according to the Sequenase kit (USB) protocol. The entire 1463 nt insert was found to be of the correct sequence, no misincorporations having resulted from the two sets of PCR reactions.

EXAMPLE 2

[0073] This objective was the humanisation of YFC51.1.1 rat anti-human -CD18 heavy and light chains. The DNA

sequence of the variable regions of both chains had been determined and is shown in

SEQ ID NOS 9 and 10 - heavy chain and
SEQ ID NOS 11 and 12 - light chain.

[0074] Using the selection procedure described in Step (2) above, the human variable domain frameworks of the NEWM heavy chain and REI light chain (Kabat et al, "Sequences of proteins of immunological interest", U.S. Dept. of Health and Human Services, U.S. Government Printing Office (1987)) were chosen for the humanisation process.

[0075] The humanised heavy and light chains were constructed as follows.

(i) Light Chain

Light chain oligonucleotide primers:

[0076]

A_L: SEQ ID NO: 13:
B_L: SEQ ID NO: 14:
C_L: SEQ ID NO: 15:
D_L: SEQ ID NO: 16:
E_L: SEQ ID NO: 17:
F_L: SEQ ID NO: 18:
G_L: SEQ ID NO: 19:
H_L: SEQ ID NO: 20:

[0077] PCR reactions were performed in a programmable heating block (Hybaid) using 20 rounds of temperature cycling (94°C for 1 min, 50°C for 2 min, and 72°C for 3 min) followed by a final 10 min step at 72°C. 1 μg of each primer, a specified amount of template, and 2.5 units of Taq polymerase (Perkin Elmer Cetus) were used in a final volume of 100 μl with the reaction buffer as recommended by the manufacturer.

[0078] The initial template for the PCR was CAMPATH-1H light chain (humanised CAMPATH-1 on REI framework; Page and Sydenham, Biotechnology 9, 64-68, (1991)). Four initial PCR reactions were carried out, with 10ng of template per reaction, using the primer pairs A_L with B_L' C_L with D_L, E_L with F_L, and G_L with H_L respectively. The products of these PCR reactions, fragments AB_L, CD_L, EF_L and GH_L respectively, were purified using Prep-A-Gene (Bio-Rad) following the protocol recommended by the manufacturer. Fragments AB_L with CD_L, and EF_L with GH_L were combined using a quarter of each purified product, and subjected to recombinant PCR reactions with primers A_L plus D_L, and E_L plus H_L respectively. The products of these reactions, fragments AD_L and EH_L, were purified as above, and a quarter of each combined in a recombinant PCR reaction using primers A_L and H_L. The final humanised light chain recombinant PCR product, AH_L, was cloned into the HindIII site of pUC-18 (BRL) following the method of Crowe et al. (1991), utilising the HindIII sites in primers A_L and H_L. Plasmid isolates were sequenced by the dideoxy chain termination method, and clones of the correct sequence chosen.

(ii) Heavy Chain

Heavy chain oligonucleotide primers:

[0079]

A_H: SEQ ID NO: 21:
B_H: SEQ ID NO: 22:
C_H: SEQ ID NO: 23:
D_H: SEQ ID NO: 24:
E_H: SEQ ID NO: 25:
F_H: SEQ ID NO: 26:
G_H: SEQ ID NO: 27:
H_H: SEQ ID NO: 28:

[0080] The initial template for the PCR was CAMPATH-1H heavy chain. The rodent CDR's were grafted on to the template using the recombinant PCR method as described in section (i) but using oligonucleotide primers A_H to H_H. The

final PCR, i.e. fragments AD_H and EH_H with primers A_H and H_H, did not give a high yield of product so a fragment AF_H was generated (from fragments AD_H and EF_H) and used with fragment EH_H in a PCR with primers A_H and H_H. Oligonucleotides A_H and H_H were designed with HindIII and EcoRI sites respectively to enable initial cloning of the humanised variable region, and a SpeI site was introduced into the NEWM framework 4 (FR4) region of oligonucleotide G_H to facilitate subsequent cloning of the variable region with a suitable constant region of choice. The SpeI site was chosen so as not to alter the leucine residue at position 109 (numbering according to Kabat et al, ibid) of the humanised heavy chain template. Four out of the six human heavy J-minigenes possess a leucine at this position; Kabat et al ibid). Thus the use of the engineered SpeI site should be generally applicable.

[0081] The humanised heavy chain variable region recombinant PCR product was cloned into HindIII/EcoRI-cut pUC-18 (BRL), and plasmid isolates of the correct sequence were chosen. The FR4 and γ1 constant regions of CAMPATH-1H heavy chain were PCR cloned into pUC-18 (BRL) using oligonucleotide primers XH (SEQ ID NO: 29) and YH (SEQ ID NO: 30). Primer X_H contains SpeI and HindIII sites, and Y_H an EcoRI site. The HindIII and EcoRI sites were used to clone the PCR product into pUC-18, and plasmid isolates of the correct sequence were selected. The complete heavy chain was subsequently reconstituted from the humanised variable region and γ1 constant region clones using the engineered FR4 SpeI site.

Sequence Listing

1. INFORMATION FOR SEQ ID NO : 1 :

i) SEQUENCE CHARACTERISTICS :
(A) LENGTH : 1457
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

ii) MOLECULE TYPE : cDNA

ix) FEATURE
(A) NAME/KEY : CDS [? CODING SEQUENCE]
(B) LOCATION : 1 1457
(D) OTHER INFORMATION : /Product = "Variable region heavy chain"
Standard name = "HUMDXCH.23"

ix) FEATURE
(A) NAME/KEY : Misc feature
(B) LOCATION : 156 182
(D) OTHER INFORMATION : /function = CAMPATH 1H FRH1

ix) FEATURE
(A) NAME/KEY : Misc feature
(B) LOCATION : 183 197
(D) OTHER INFORMATION : /function = CAMPATH 1H CDRH1

ix) FEATURE
(A) NAME/KEY : Misc feature
(B) LOCATION : 198 224
(D) OTHER INFORMATION : /function = CAMPATH 1H FRH2

xi) SEQUENCE DESCRIPTION : SEQ ID NO : 1 :

```
        AAGCTTTACA GTTACTGAGC ACACAGGACC TCACC ATG          38
                                                  Met
        ....................................              155

TGC ACC GTG TCT GGC TTC ACC TTC ACC GAT TTC TAC ATG AAC   197
Cys Thr Val Ser Gly Phe Thr Phe Thr Trp Phe Tyr Met Asn

        TGG GTG AGA CAG CCA CCT GGA CGA GGT                224
        Trp Val Arg Gln Pro Pro Gly Arg Gly
        ....................................              1430
        CCG GGT AAA TGAGTGCGAC GGAAGCTT                    1547
        Pro Gly Lys
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2. INFORMATION FOR SEQ ID NO : 2 :

i) SEQUENCE CHARACTERISTICS :
   (A) LENGTH : 24 base pairs
   (B) TYPE : nucleic acid
   (C) STRANDEDNESS : single
   (D) TOPOLOGY : linear

ii) MOLECULE TYPE : ssDNA
iii) HYPOTHETICAL : No
iv) ANTI-SENSE : No

xi) SEQUENCE DESCRIPTION : SEQ ID NO : 2 :

```
        GATCAAGCTT TACAGTTACT GAGC                         24
```

3. INFORMATION FOR SEQ ID NO : 3 :

i) SEQUENCE CHARACTERISTICS :
   (A) LENGTH : 45 base pairs
   (B) TYPE : nucleic acid
   (C) STRANDEDNESS : single
   (D) TOPOLOGY : linear

ii) MOLECULE TYPE : ssDNA
iii) HYPOTHETICAL : No
iv) ANTI-SENSE : Yes

xi) SEQUENCE DESCRIPTION : SEQ ID NO : 3 :

TGGCACAGAC CGTCGTGGAA GTCGTGAATA CCATACCCAC ACCCG 45

4. INFORMATION FOR SEQ ID NO : 4 :

i) SEQUENCE CHARACTERISTICS :
(A) LENGTH : 45 base pairs
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

ii) MOLECULE TYPE : ssDNA
iii) HYPOTHETICAL : No
iv) ANTI-SENSE : No

xi) SEQUENCE DESCRIPTION : SEQ ID NO : 4 :

ACTTATGGTA TGGGTGTGGG CTGGGTGAGA CAGCCACCTG GACGA 45

5. INFORMATION FOR SEQ ID NO : 5 :

i) SEQUENCE CHARACTERISTICS :
(A) LENGTH : 27 base pairs
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

ii) MOLECULE TYPE : ssDNA
iii) HYPOTHETICAL : No
iv) ANTI-SENSE : Yes

xi) SEQUENCE DESCRIPTION : SEQ ID NO : 5 :

CATTTACTCA CGCTGCCTTC GAACTAG 27

6. INFORMATION FOR SEQ ID NO : 6 :

i) SEQUENCE CHARACTERISTICS :
(A) LENGTH : 207 base pairs
(B) TYPE : nucleic acid
(C) STRANDEDNESS : double
(D) TOPOLOGY : linear

ii) MOLECULE TYPE : dsDNA

xi) SEQUENCE DESCRIPTION : SEQ ID NO : 6 :

GATCAAGCTT TACAGTTACT GAGCACACAG GACCTCACCA TG 42
..... 159
TGCACCGTGT CTGGCAGCAC CTTCAGCACT TATGGTATGG GTGTGGGC 207

7. INFORMATION FOR SEQ ID NO : 7 :

i) SEQUENCE CHARACTERISTICS :
(A) LENGTH : 1285
(B) TYPE : nucleic acid
(C) STRANDEDNESS : double
(D) TOPOLOGY : linear

ii) MOLECULE TYPE : dsDNA

xi) SEQUENCE DESCRIPTION : SEQ ID NO : 7 :

ACTTATGGTA TGGGTGTGGG CTGGGTGAGA CAGCCACCTG GACGAGGT 48
..... 1254
CCGGGTAAAT GAGTGCGACG GAAGCTTGAT C 1285

8. INFORMATION FOR SEQ ID NO : 8 :

i) SEQUENCE CHARACTERISTICS :
(A) LENGTH : 1471
(B) TYPE : nucleic acid
(C) STRANDEDNESS : double
(D) TOPOLOGY : linear

ii) MOLECULE TYPE : dsDNA

ix) FEATURE
(A) NAME/KEY : CDS [?]
(B) LOCATION : 1 1471
(D) OTHER INFORMATION : /PRODUCT = "Variable region heavy chain"

ix) FEATURE
(A) NAME/KEY : misc feature
(B) LOCATION : 160 186
(D) OTHER INFORMATION : /function CAMPATH 1H FRH1

ix) FEATURE
(A) NAME/KEY : Misc feature
(B) LOCATION : 175 177
(D) OTHER INFORMATION : point mutation

ix) FEATURE
(A) NAME/KEY : Misc feature
(B) LOCATION : 184 186
(D) OTHER INFORMATION : point mutation

ix) FEATURE
(A) NAME/KEY : Misc feature
(B) LOCATION : 187 207
(D) OTHER INFORMATION : /function -DK48 CDRH1

ix) FEATURE
(A) NAME/KEY : Misc feature
(B) LOCATION : 208 234
(D) OTHER INFORMATION : /function CAMPATH 1H FRH 2

xi) SEQUENCE DESCRIPTION : SEQ ID NO : 8 :

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GATCAAGCTT TACAGTTACT GAGCACACAG GACCTCACC ATG      42
                                           Met
.....                                              159

TGC ACC GTG TCT GGC AGC ACC TTC AGC ACT TAT GGT ATG  198
Cys Thr Val Ser Gly (Ser) Thr Phe (Ser) Tur Tyr Gly Met

GGT GTG GGC TGG GTG AGA CAG CCA CCT GGA CGA GGT     234
Gly Val Gly Trp Val Arg Gln Pro Pro Gly Arg Gly
.....                                             1440

CCG GGT AAA TGAGTGCGAC GGAAGCTTGA TC               1471
Pro Gly Ala
```

(9) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 417 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Rattus rattus

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..417
(D) OTHER INFORMATION: /product= "Heavy chain variable region with signal sequence"
/standard_name= "YFC51.1.1"

(ix) FEATURE:
(A) NAME/KEY: misc_signal
(B) LOCATION: 1..57
(D) OTHER INFORMATION: /function= "Signal sequence"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 148..162
(D) OTHER INFORMATION: /function= "CDR 1"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 205..255

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 352..384

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

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ATG AAA TGC AGC TGG ATC AAC CTC TTC TTG ATG GCA CTA GCT TCA GGG        48
Met Lys Cys Ser Trp Ile Asn Leu Phe Leu Met Ala Leu Ala Ser Gly
 1               5                  10                  15

GTC TAC GCA GAA GTG CAG CTG CAA CAG TCT GGG CCC GAG CTT CGG AGA        96
Val Tyr Ala Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Arg Arg
            20                  25                  30

CCT GGG TCC TCA GTC AAG TTG TCT TGT AAG ACT TCT GGC TAC AGC ATT       144
Pro Gly Ser Ser Val Lys Leu Ser Cys Lys Thr Ser Gly Tyr Ser Ile
        35                  40                  45
```

```
AAA GAT TAC CTT CTG CAC TGG GTA AAA CAT AGG CCA GAA TAC GGC CTG      192
Lys Asp Tyr Leu Leu His Trp Val Lys His Arg Pro Glu Tyr Gly Leu
     50                  55                  60

GAA TGG ATA GGA TGG ATT GAT CCT GAG GAT GGT GAA ACA AAG TAT GGT      240
Glu Trp Ile Gly Trp Ile Asp Pro Glu Asp Gly Glu Thr Lys Tyr Gly
 65                  70                  75                  80

CAG AAG TTT CAA AGC AGG GCC ACA CTC ACT GCA GAT ACA TCC TCC AAC      288
Gln Lys Phe Gln Ser Arg Ala Thr Leu Thr Ala Asp Thr Ser Ser Asn
                 85                  90                  95

ACA GCC TAC ATG CAA CTC AGC AGC CTG ACG TCT GAC GAC ACA GCA ACC      336
Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Asp Asp Thr Ala Thr
            100                 105                 110

TAT TTT TGT ACT AGA GGC GAA TAT AGA TAC AAC TCG TGG TTT GAT TAC      384
Tyr Phe Cys Thr Arg Gly Glu Tyr Arg Tyr Asn Ser Trp Phe Asp Tyr
        115                 120                 125

TGG GGC CAA GGC ACT CTG GTC ACT GTC TCT TCA                          417
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
    130                 135
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(10) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 139 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

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 1               5                  10                  15

Val Tyr Ala Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Arg Arg
            20                  25                  30

Pro Gly Ser Ser Val Lys Leu Ser Cys Lys Thr Ser Gly Tyr Ser Ile
        35                  40                  45

Lys Asp Tyr Leu Leu His Trp Val Lys His Arg Pro Glu Tyr Gly Leu
     50                  55                  60

Glu Trp Ile Gly Trp Ile Asp Pro Glu Asp Gly Glu Thr Lys Tyr Gly
 65                  70                  75                  80

Gln Lys Phe Gln Ser Arg Ala Thr Leu Thr Ala Asp Thr Ser Ser Asn
                 85                  90                  95
```

Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Asp Asp Thr Ala Thr
100 105 110

Tyr Phe Cys Thr Arg Gly Glu Tyr Arg Tyr Asn Ser Trp Phe Asp Tyr
115 120 125

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
130 135

(11) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 375 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Rattus rattus

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..375
(D) OTHER INFORMATION: /product= "Variable region light chain"
/standard_name= "YPC51.1.1"

(ix) FEATURE:
(A) NAME/KEY: misc_signal
(B) LOCATION: 1..60
(D) OTHER INFORMATION: /function= "Signal sequence"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 130..162
(D) OTHER INFORMATION: /function= "CDR 1"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 208..228
(D) OTHER INFORMATION: /function= "CDR 2"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 325..351
(D) OTHER INFORMATION: /function= "CDR 3"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

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 1               5                  10                  15

GGT GCC CAG TGT GAT GTC CAG ATG ACC CAG TCT CCG TCT TAT CTT GCT        96
Gly Ala Gln Cys Asp Val Gln Met Thr Gln Ser Pro Ser Tyr Leu Ala
            20                  25                  30
```

```
GCG TCT CCT GGA GAA AGT GTT TCC ATC AGT TGC AAG GCA AGT AAG AGC      144
Ala Ser Pro Gly Glu Ser Val Ser Ile Ser Cys Lys Ala Ser Lys Ser
        35                  40                  45

ATT AGC AAT TAT TTA GCC TGG TAT CAA CAG AAA CCT GGG GAA GCA AAT      192
Ile Ser Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Glu Ala Asn
    50                  55                  60

AAA CTT CTT GTC TAT TAT GGG TCA ACT TTG CGA TCT GGA ATT CCA TCG      240
Lys Leu Leu Val Tyr Tyr Gly Ser Thr Leu Arg Ser Gly Ile Pro Ser
65                  70                  75                  80

AGG TTC AGT GGC AGT GGA TCT GGT ACA GAT TTC ACT CTC ACC ATC AGA      288
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Arg
                85                  90                  95

AAC CTG GAG CCT GCA GAT TTT GCA GTC TAC TAC TGT CAA CAG TAT TAT      336
Asn Leu Glu Pro Ala Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Tyr
            100                 105                 110

GAA AGA CCG CTC ACG TTC GGT TCT GGG ACC AAG CTG GAG                  375
Glu Arg Pro Leu Thr Phe Gly Ser Gly Thr Lys Leu Glu
        115                 120                 125
```

(12) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 125 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12

```
Met Arg Val Gln Val Gln Phe Leu Gly Leu Leu Leu Trp Thr Ser
 1               5                  10                  15

Gly Ala Gln Cys Asp Val Gln Met Thr Gln Ser Pro Ser Tyr Leu Ala
            20                  25                  30

Ala Ser Pro Gly Glu Ser Val Ser Ile Ser Cys Lys Ala Ser Lys Ser
        35                  40                  45

Ile Ser Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Glu Ala Asn
    50                  55                  60

Lys Leu Leu Val Tyr Tyr Gly Ser Thr Leu Arg Ser Gly Ile Pro Ser
65                  70                  75                  80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Arg
                85                  90                  95
```

```
Asn Leu Glu Pro Ala Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Tyr
            100                 105                 110

Glu Arg Pro Leu Thr Phe Gly Ser Gly Thr Lys Leu Glu
        115                 120                 125
```

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Rattus rattus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

AGTGGNTAGA CAGATGGGGC 20

(13) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: ssDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GATCAAGCTT CTCTACAGTT ACTGAGCACA 30

(14) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: ssDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GCTAAATAAT TGCTAATGCT CTTACTTGCT TTACAGGTGA TGG 43

(15) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: ssDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

AGAGCATTAG CAATTATTTA GCCTGGTACC AGCAGAAGCC AGG 43

(16) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: ssDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

AGATCGCAAA GTTGACCCAT AGTAGATCAG CAGCTTTGGA G 41

(17) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: ssDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

TATGGGTCAA CTTTGCGATC TGGTGTGCCA AGCAGATTCA G 41

(18) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 47 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: ssDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CGTGAGCGGT CTTTCATAAT ACTGTTGGCA GTAGTAGGTG GCGATGT 47

(19) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 47 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: ssDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CAACAGTATT ATGAAAGACC GCTCACGTTC GGCCAAGGGA CCAAGGT 47

(20) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: ssDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GATCAAGCTT CTAACACTCT CCCCTGTTGA 30

(21) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: ssDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TGGGATCGAT CAAGCTTTAC AGTTACTGAG C 31

(22) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: ssDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GTGCAGAAGG TAATCGGTGA AGGTGAAGCC AGACAC 36

(23) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: ssDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GATTACCTTC TGCACTGGGT GAGACAGCCA CCTGGA 36

(24) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 54 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: ssDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

ATACTTTGTT TCACCATCCT CAGGATCAAT CCATCCAATC CACTCAAGAC CTCG 54

(25) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 54 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: ssDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GGTGAAACAA AGTATGGTCA GAAGTTTCAA AGCAGAGTGA CAATGCTGGT AGAC 54

(26) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: ssDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CCACGAGTTG TATCTATATT CGCCTCTTGC ACAATAATAG ACCGC 45

(27) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 54 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: ssDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

AGATACAACT CGTGGTTTGA TTACTGGGGT CAAGGCTCAC TAGTCACAGT CTCC 54

(28) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: ssDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

TAGAGTCCTG AGGGAATTCG GACAGCCGGG AAGGTG 36

(29) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: ssDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GCTGCTCCTT TTAAGCTTTG GGGTCAAGGC TCACTAGTCA CAGTCTCC 48

(30) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: ssDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

AAGCTTCCGT CGAATTCATT TACCCGGAGA CAG 33

Claims

1. A method for producing a double- or single-stranded DNA of formula

5' F1-M-F2 3'

encoding an antibody chain or fragment thereof in which at least one of the complementarity determining regions (CDRs) of the variable region of the antibody chain is derived from a first mammalian antibody, and the framework of the variable region is derived from a second, different mammalian antibody, wherein M comprises DNA encoding a CDR of the first antibody and F1 and F2 respectively encode 5' and 3' sequences flanking M, which method comprises;

(i) preparing a single- or double-stranded DNA template of the formula

5' f1-H-f2 3'

wherein H comprises DNA encoding a CDR of a different specificity from M and f1 and f2 are substantially homologous to F1 and F2 respectively (i.e. f1 and f2 may have minor changes compared to F1 and F2 respectively)

(ii) obtaining DNA oligonucleotide primers A, B, C and D wherein

A

- comprises a sequence a^1 which has a 5' end corresponding to the 5' end of F1 and which is identical to a corresponding length (i.e. a sequence of the same number of nucleotides and with the identical or complementary sequence) of the sequence F1, is oriented in a 5' to 3' direction towards H;

B consists of the sequence

5' b^1-b^2 3'

wherein

- b^1 comprises a sequence complementary to a corresponding length of M and has a 3' end which is complementary to the 5' end of M, and
- b^2 is complementary to a sequence of corresponding length in F^1 and has a 5' end which starts at the nucleotide complementary to the 3' end of F^1;

C consists of the sequence

5' c^1-c^2 3'

wherein

- c^1 comprises a sequence identical to the corresponding length of M and has a 3' end which corresponds to the 3' end of M, and
- c^2 is identical to a sequence of corresponding length in F2 and has a 5' end which starts at the nucleotide corresponding to the 5' end of F2;

D

- comprises a sequence d^1 which has a 5' end complementary to the 3' end of F2 and which is complementary to a corresponding length of F2, and
- is oriented in a 5' to 3' direction towards H;

and wherein b^1 and c^1 overlap by a length sufficient to permit annealing of their 5' ends to each other under conditions which allow a polymerase chain reaction (PCR) to be performed;

(iii) performing, in any desired order, PCR reactions with primer pairs A,B and C,D on the template prepared in (i) above; and

(iv) mixing the products obtained in (iii) above and performing a PCR reaction using primers A and D.

2. A method according to any one of the preceding claims wherein the primers A and D contain at least one restriction endonuclease recognition site within 10 nucleotides of their 5' ends.

3. A method according to any one of the preceding claims wherein, in the primers B and C, b^1 and c^1 are the same number of nucleotides in length.

4. A method according to any one of the preceding claims wherein primers A, B, C and D are each from 15 to 200 nucleotides in length.

5. A method for the production of a humanised antibody wherein at least one of the CDR regions of a human antibody light or heavy chain is replaced by a method according to any one of the preceding claims.

6. A method according to any one of the preceding claims which further includes the introduction of the DNA obtained into an expression vector.

7. A method according to claim 6 which further includes the introduction of the expression vector into a host cell.

8. A method according to claim 7 which further includes expression of the DNA obtained and recovery of the expressed product.

9. A method for producing a double- or single-stranded DNA of formula

5' F1-M1-F2-M2-F3-M3-F4 3'

encoding an antibody chain or fragment thereof in which the three complementarity determining regions (CDRs) of the variable region of the antibody chain are derived from a first mammalian antibody, and the tour framework regions of the variable domain are derived from a second, different mammalian antibody, wherein M1, M2 and MS comprise DNA encoding CDRs of the first antibody and F1, F2, F3 and F4 comprise framework sequences flanking the CDRs M1, M2 and M3, which method comprises;

(i) preparing a single- or double-stranded DNA template of the formula

5' f1-H1-f2-H2-f3-H3-f4 3'

wherein H1, H2 and H3 comprises DNA encoding CDRs of a different specificity from M1, M2 and M3, and f1, f2, f3 and f4 are substantially homologous to F1, F2, F3 and F4 respectively (i.e. f1, f2, f3 and f4 may have minor changes compared to F1, F2, F3 and F4 respectively)

(ii) obtaining DNA oligonucleotide primers A, B, C, D, E, F, G and H wherein

A

- comprises a sequence a^1 which has a 5' end corresponding to the 5' end of F1 and which is identical to a corresponding length of the sequence F1,

- is oriented in a 5' to 3' direction towards H1;

B consists of the sequence

5' b^1-b^2 3'

wherein

- b^1 comprises a sequence complementary to a corresponding length of M1 and has a 3' end which is complementary to the 5' end of M1, and

- b^2 is complementary to a sequence of corresponding length (i.e. a sequence of the same number of nucleotides and with the identical or complementary sequence) in F^1 and has a 5' end which starts at the nucleotide complementary to the 3' end of F1;

C consists of the sequence

5' c^1-c^2 3'

wherein

- c^1 comprises a sequence identical to the corresponding length of M^1 and has a 3' end which corre-

sponds to the 3' end of M^1, and

- c^2 is identical to a sequence of corresponding length in F2 and has a 5' end which starts at the nucleotide corresponding to the 5' end F2;

D consists of the sequence

5' d^1-d^2 3'

wherein

- d^1 comprises a sequence complementary to a corresponding length of M2 and has a 3' end which is complementary to the 5' end of M2, and
- d^2 is complementary to a sequence of corresponding length in F2 and has a 5' end which starts at the nucleotide complementary to the 3' end of F2;

E consists of the sequence

5' e^1-e^2 3'

wherein

- e^1 comprises a sequence identical to the corresponding length of M2 and has a 3' end which corresponds to the 3' end of M2, and
- e^2 is identical to a sequence of corresponding length in F3 and has a 5' end which starts at the nucleotide corresponding to the 5' end F3;

F consists of the sequence

5' f^1-f^2 3'

wherein

- f^1 comprises a sequence complementary to a corresponding length of M3 and has a 3' end which is complementary to the 5 ' end of M3, and
- f^2 is complementary to a sequence of corresponding length in F3 and has a 5' end which starts at the nucleotide complementary to the 3' end of F3;

G consists of the sequence

5' g^1-g^2 3'

wherein

- g^1 comprises a sequence identical to the corresponding length of M3 and has a 3' end which corresponds to the 3' end of M3, and
- g^2 is identical to a sequence of corresponding length in F4 and has a 5' end which starts at the nucleotide corresponding to the 5' end F4;

H

- comprises a sequence h^1 which has a 5' end complementary to the 3' end of F4 and which is complementary to a corresponding length of F4, and
- is oriented in a 5' to 3' direction towards H3;

and wherein the pairs b^1 and c^1, d^1 and e^1, and f^1 and g^1 overlap by a length sufficient to permit annealing of their 5' ends to each other under conditions which allow a PCR to be performed;

(iii) performing, in any desired order, PCR reactions with primer pairs A,B; C,D; E,F; and G,H on the template

prepared in (i) above to obtain DNA fragments AB, CD, EF and GH; and

(iv) splicing the fragments obtained in (iii) above to obtain the desired DNA.

Patentansprüche

1. Verfahren zur Herstellung einer doppel- oder einsträngigen DNA der Formel

5' F1-M-F2 3'

die eine Antikörperkette oder ein Fragment davon kodiert, worin wenigstens eine der komplementaritätsbestimmenden Regionen ("complementarity determining regions", CDRs) der variablen Region der Antikörperkette aus einem ersten Säugetier-Antikörper stammt und das Gerüst der variablen Region aus einem zweiten unterschiedlichen Säugetier-Antikörper stammt, worin M DNA umfaßt, die eine CDR des ersten Antikörpers kodiert, und F1 und F2 5'- bzw. 3'-Sequenzen kodieren, die M flankieren, wobei das Verfahren umfaßt:

(i) Herstellen einer ein- oder doppelsträngigen DNA-Schablone der Formel

5' f1-H-f2 3'

worin H DNA umfaßt, die eine CDR einer unterschiedlichen Spezifizität von M kodiert, und f1 und f2 im wesentlichen homolog zu F1 bzw. F2 sind (d.h. f1 und f2 können geringfügige Änderungen im Vergleich zu F1 bzw. F2 aufweisen)

(ii) Erhalten der DNA-Oligonukleotid-Primer A, B, C und D, worin

A

- eine Sequenz a^1 umfaßt, die ein dem 5'-Ende von F1 entsprechendes 5'-Ende aufweist und die indentisch mit einer entsprechenden Länge (d.h. mit einer Sequenz der gleichen Anzahl von Nukleotiden und mit der identischen oder komplementären Sequenz) der Sequenz F1 ist,

- in einer 5'-zu-3'-Richtung zu H orientiert ist;

B aus der Sequenz

5' b^1-b^2 3'

besteht, worin

- b^1 eine zu einer entsprechenden Länge von M komplementäre Sequenz umfaßt und ein 3'-Ende aufweist, das komplementär zum 5'-Ende von M ist, und

- b^2 komplementär zu einer Sequenz entsprechender Länge in F1 ist und ein 5'-Ende aufweist, das am zum 3'-Ende von F1 komplementären Nukleotid beginnt;

C aus der Sequenz

5' c^1-c^2 3'

besteht, worin

- c^1 eine mit der entsprechenden Länge von M indentische Sequenz umfaßt und ein 3'-Ende aufweist, das dem 3'-Ende von M entspricht, und
- c^2 identisch mit einer Sequenz entsprechender Länge in F2 ist und ein 5'-Ende aufweist, das am dem 5'-Ende von F2 entsprechenden Nukleotid beginnt;

D

- eine Sequenz d^1 umfaßt, die ein zum 3'-Ende von F2 komplementäres 5'-Ende aufweist und die komplementär zu einer entsprechenden Länge von F2 ist, und

- in einer 5'-zu-3'-Richtung zu H orientiert ist;

und worin b^1 und c^1 mit einer ausreichenden Länge zur Verschmelzung ihrer 5'-Enden miteinander unter Bedingungen überlappen, die die Durchführung einer PCR erlauben;

(iii) Durchführen von PCR-Reaktionen in jeder gewünschten Reihenfolge mit den Primerpaaren A,B und C,D an der in (i) oben hergestellten Schablone; und

(iv) Mischen der in (iii) oben erhaltenen Produkte und Durchführen einer PCR-Reaktion unter Verwendung der Primer A und D.

2. Verfahren gemäß einem der vorhergehenden Ansprüche, worin die Primer A und D wenigstens eine Restriktionsendonuklease-Erkennungsstelle innerhalb 10 Nukleotiden ihrer 5'-Enden enthalten.

3. Verfahren gemäß einem der vorhergehenden Ansprüche, worin in den Primern B und C b^1 und c^1 in der Länge die gleiche Anzahl von Nukleotiden aufweisen.

4. Verfahren gemäß einem der vorhergehenden Ansprüche, worin die Primer A, B, C und D jeweils eine Länge von 15 bis 200 Nukleotiden aufweisen.

5. Verfahren zur Herstellung eines humanisierten Antikörpers, worin wenigsten eine der CDR-Regionen einer leichten oder schweren Kette eines menschlichen Antikörpers durch ein Verfahren gemäß einem der vorhergehenden Ansprüche ersetzt wird.

6. Verfahren gemäß einem der vorhergehenden Ansprüche, das zusätzlich die Einführung der erhaltenen DNA in einen Expressionsvektor einschließt.

7. Verfahren gemäß Anspruch 6, das zusätzlich die Einführung des Expressionsvektors in eine Wirtszelle einschließt.

8. Verfahren gemäß Anspruch 7, das zusätzlich die Expression der erhaltenen DNA und Gewinnung des exprimierten Produkts einschließt.

9. Verfahren zur Herstellung einer doppel- oder einsträngigen DNA der Formel

5' F1-M1-F2-M2-F3-M3-F4 3'

die eine Antikörperkette oder ein Fragment davon kodiert, worin die drei komplementaritätsbestimmenden Regionen ("complementarity determining regions", CDRs) der variablen Region der Antikörperkette aus einem ersten Säugetier-Antikörper stammen und die vier Gerüstregionen der variablen Domäne aus einem zweiten unterschiedlichen Säugetier-Antikörper stammen, worin M1, M2 und M3 DNA umfassen, die CDRs des ersten Antikörpers kodiert, und F1, F2, F3 und F4 Gerüstsequenzen umfassen, die die CDRs M1, M2 und M3 flankieren, wobei das Verfahren umfaßt:

(i) Herstellen einer ein- oder doppelsträngigen DNA-Schablone der Formel

5' f1-H1-f2-H2-f3-H3-f4 3'

worin H1, H2 und H3 DNA umfaßt, die CDRs einer unterschiedlichen Spezifizität von M2, M2 und M3 kodiert, und f1, f2, f3 und f4 im wesentlichen homolog zu F1, F2, F3 bzw. F4 sind (d.h. f1, f2, f3 und f4 können geringfügige Änderungen im Vergleich zu F1, F2, F3 bzw. F4 aufweisen)

(ii) Erhalten von DNA-Oligonukleotid-Primern A, B, C, D, E, F, G und H, worin

A

- eine Sequenz a^1 umfaßt, die ein dem 5'-Ende von F1 entsprechendes 5'-Ende aufweist und die identisch mit einer entsprechenden Länge der Sequenz F1 ist,

- in einer 5'-zu-3'-Richtung zu H1 orientiert ist;

B aus der Sequenz

$5'\ b^1\text{-}b^2\ 3'$

besteht, worin

- b^1 eine zu einer entsprechenden Länge von M1 komplementäre Sequenz umfaßt und ein 3'-Ende aufweist, das komplementär zum 5'-Ende von M1 ist, und

- b^2 komplementär zu einer Sequenz entsprechender Länge (d.h. mit einer Sequenz der gleichen Anzahl von Nukleotiden und mit der identischen oder komplementären Sequenz) in F1 ist und ein 5'-Ende aufweist, das am zum 3'-Ende von F1 komplementären Nukleotid beginnt;

C aus der Sequenz

$5'\ c^1\text{-}c^2\ 3'$

besteht, worin

- c^1 eine mit der entsprechenden Länge von M1 identische Sequenz umfaßt und ein 3'-Ende aufweist, das dem 3'-Ende von M1 entspricht, und

- c^2 identisch mit einer Sequenz entsprechender Länge in F2 ist und ein 5'-Ende aufweist, das am dem 5'-Ende von F2 entsprechenden Nukleotid beginnt;

D aus der Sequenz

$5'\ d^1\text{-}d^2\ 3'$

besteht, worin

- d^1 eine zu einer entsprechenden Länge von M2 komplentäre Sequenz umfaßt und ein 3'-Ende aufweist, das komplementär zum 5'-Ende von M2 ist, und
- d^2 komplementär zur einer Sequenz entsprechender Länge in F2 ist und ein 5'-Ende aufweist, das am zum 3'-Ende von F2 komplementären Nukleotid beginnt;

E aus der Sequenz

$5'\ e^1\text{-}e^2\ 3'$

besteht, worin

- e^1 eine mit der entsprechenden Länge von M2 indentische Sequenz umfaßt und ein 3'-Ende aufweist, das dem 3'-Ende von M2 entspricht, und

- e^2 identisch mit einer Sequenz entsprechender Länge in F3 ist und ein 5'-Ende aufweist, das am dem 5'-Ende von F3 entsprechenden Nukleotid beginnt;

F aus der Sequenz

$5'\ f^1\text{-}f^2\ 3'$

besteht, worin

- f^1 eine zu einer entsprechenden Länge von M3 komplementäre Sequenz umfaßt und ein 3'-Ende aufweist, das komplementär zum 5'-Ende von M3 ist, und

- f^2 komplementär zu einer Sequenz entsprechender Länge in F3 ist und ein 5'-Ende aufweist, das am zum 3'-Ende von F3 komplementären Nukleotid beginnt;

G aus der Sequenz

5' g^1-g^2 3'

besteht, worin

- g^1 eine mit der entsprechenden Länge von M3 identische Sequenz umfaßt und ein 3'-Ende aufweist, das dem 3'-Ende von M3 entspricht, und

- g^2 identisch mit einer Sequenz entsprechender Länge in F4 ist und ein 5'-Ende aufweist, das am dem 5'-Ende von F4 entsprechenden Nukleotid beginnt;

H

- eine Sequenz h^1 umfaßt, die ein zum 3'-Ende von F4 komplementäres 5'-Ende aufweist und die komplementär zu einer entsprechenden Länge von F4 ist, und

- in einer 5'-zu-3'-Richtung zu H3 orientiert ist;

und worin die Paare b^1 und c^1, d^1 und e^1, und f^1 und g^1 mit einer zum Verschmelzen ihrer 5'-Enden miteinander ausreichenden Länger unter Bedingungen überlappen, die die Durchführung einer PCR erlauben;

(iii) Durchführen von PCR-Reaktionen in jeder gewünschten Reihenfolge mit den Primer-Paaren A,B; D,D; E,F; und G,H an der in (i) oben hergestellten Schablone, um DNA-Fragmente AB, CD, EF und GH zu erhalten; und

(iv) Spleißen der in (iii) oben erhaltenen Fragmente, um die gewünschte DNA zu erhalten.

Revendications

1. Méthode de production d'un ADN double brin ou simple brin de formule

5' F1-M-F2 3'

codant pour une chaîne d'anticorps ou un fragment de celle-ci où au moins l'une des régions complémentaires déterminantes (CDR) de la région variable de la chaîne d'anticorps est dérivée d'un premier anticorps de mammifère, et le motif de la région variable dérivé d'un deuxième anticorps de mammifère différent, où M comprend de l'ADN codant pour une CDR du premier anticorps, et F1 et F2 codent pour des séquences bordant M, laquelle méthode comprend:

(i) la préparation d'une matrice d'ADN simple brin ou double brin de formule

5' f1-H-f2 3'

dans laquelle H comprend un ADN codant pour une CDR d'une spécificité différente de M, et f1 et f2 sont essentiellement homologues à F1 et F2 respectivement (c'est-à-dire que f1 et f2 peuvent présenter des variations mineures vis-à-vis de F1 et F2 respectivement)
(ii) l'obtention d'amorces d'oligonucléotides d'ADN A, B, C et D où

A

- comprend une séquence a^1 qui possède une extrémité 5' correspondant à l'extrémité 5' de F1 et qui

est identique à une longueur correspondante (c'est-à-dire une séquence comportant le même nombre de nucléotides et présentant la séquence identique ou complémentaire) de la séquence F1,

- est orientée en sens 5' - 3' vers H;

B consiste en la séquence

5' b^1-b^2 3'

dans laquelle

- b^1 comprend une séquence complémentaire à une longueur correspondante de M et possède une extrémité 3' complémentaire à l'extrémité 5' de M, et
- b^2 est complémentaire à une séquence de longueur correspondante dans F1 et possède une extrémité 5' qui commence au nucléotide complémentaire à l'extrémité 3' de F1;

C consiste en la séquence

5' c^1-c^2 3'

dans laquelle

- c^1 comprend une séquence identique à la longueur correspondante de M et possède une extrémité 3' correspondant à l'extrémité 3' de M, et
- c^2 est identique à une séquence de longueur correspondante dans F2 et possède une extrémité 5' qui commence au nucléotide correspondant à l'extrémité 5' de F2;

D

- comprend une séquence d^1 possédant une extrémité 5' complémentaire à l'extrémité 3' de F2 et qui est complémentaire à une longueur correspondante de F2, et
- est orientée dans le sens 5' - 3' vers H;

et où b^1 et c^1 se chevauchent d'une longueur suffisante pour permettre l'annellation de leurs extrémités 5' l'une à l'autre dans des conditions permettant l'exécution d'une réaction PCR;

(iii) l'exécution, dans n'importe quel ordre souhaité, de réactions PCR avec des paires d'amorces A,B et C,D sur la matrice préparée au point (i) ci-dessus, et
(iv) le mélange des produits obtenus au point (iii) ci-dessus et l'exécution d'une réaction PCR en utilisant des amorces A et D.

2. Méthode selon l'une quelconque des revendications qui précèdent, dans laquelle les amorces A et D contiennent au moins un site de reconnaissance d'endonucléase de restriction dans les 10 nucléotides de leur extrémité 5'.

3. Méthode selon l'une quelconque des revendications qui précèdent, dans laquelle les amorces B et C, b^1 et c^1 possèdent le même nombre de nucléotides en longueur.

4. Méthode selon l'une quelconque des revendications qui précèdent, dans laquelle les amorces A, B, C et D possèdent chacune de 15 à 200 nucléotides en longueur.

5. Méthode de production d'un anticorps humanisé dans lequel au moins une des régions CDR d'une chaîne légère ou lourde d'anticorps humains est remplacé par une méthode selon l'une quelconque des revendications qui précèdent.

6. Méthode selon l'une quelconque des revendications qui précèdent, comprenant en outre l'insertion de l'ADN obtenu dans un vecteur d'expression.

7. Méthode selon la revendication 6, comprenant en outre l'introduction du vecteur d'expression dans une cellule hôte.

8. Méthode selon la revendication 7, comprenant en outre l'expression de l'ADN obtenu et le récupération du produit exprimé.

9. Méthode de production d'un ADN double brin ou simple brin de formule

5' F1-M1-F2-M2-F3-M3-F4 3'

codant pour une chaîne d'anticorps ou un fragment de celle-ci où les trois régions complémentaires déterminantes (CDR) de la région variable de la chaîne d'anticorps sont dérivées d'un premier anticorps de mammifère, et les quatre régions du motif du domaine variable sont dérivées d'un deuxième anticorps de mammifère différent, où M1, M2 et M3 comprennent de l'ADN codant pour des CDR du premier anticorps et F1, F2, F3 et F4 comprennent des séquences du motif bordant les CDR M1, M2 et M3, laquelle méthode comprend:

(i) la préparation d'une matrice d'ADN simple brin ou double brin de formule

5' f1-H1-f2-H2-f3-H3-f4 3'

dans laquelle H1, H2 et H3 comprennent de l'ADN codant pour des CDR d'une spécificité différente de M1, M2 et M3, et f1, f2, f3 et f4 sont essentiellement homologues à F1, F2, F3 et F4 respectivement (c'est-à-dire que f1 et f2 peuvent présenter des variations mineures vis-à-vis de F1 et F2 respectivement).
(ii) l'obtention d'amorces d'oligonucléotides d'ADN A, B, C, D, E, F, G et H dans lesquelles

A

- comprend une séquence a^1 qui possède une extrémité 5' correspondant à l'extrémité 5' de F1 et qui est identique à une longueur correspondante de la séquence F1,
- est orientée on sens 5' - 3' vers H;

B consiste en la séquence

5' b^1-b^2 3'

dans laquelle

- b^1 comprend une séquence complémentaire à une longueur correspondante de M1 et possède une extrémité 3' complémentaire à l'extrémité 5' de M1, et
- b^2 est complémentaire à une séquence de longueur correspondante dans F1 et possède une extrémité 5' qui commence au nucléotide complémentaire à l'extrémité 3' de F1;

C consiste en la séquence

5' c^1-c^2 3'

dans laquelle

- c^1 comprend une séquence identique à la longueur correspondante de M1 et possède une extrémité 3' correspondant à l'extrémité 3' de M1, et
- c^2 est identique à une séquence de longueur correspondante dans F2 et possède une extrémité 5' qui commence au nucléotide correspondant à l'extrémité 5' de F2;

D

- consiste en la séquence

5' d^1-d^2 3'

dans laquelle

- d^1 comprend une séquence complémentaire à une longueur correspondante de M2 et possède une extrémité 3' complémentaire à l'extrémité 5' de M2, et
- d^2 est complémentaire à une séquence de longueur correspondant dans F2 et possède une extrémité 5' qui commence au nucléotide complémentaire à l'extrémité 3' de F2;

E consiste en la séquence

5' e^1-e^2 3'

dans laquelle

- e^1 comprend une séquence identique à la longueur correspondante de M2 et possède une extrémité 3' correspondant à l'extrémité 3' de M2, et
- e^2 est identique à une séquence de longueur correspondante dans F3 et possède une extrémité 5' qui commence au nucléotide correspondant à l'extrémité 5' de F3;

F consiste en la séquence

5' f^1-f^2 3'

dans laquelle

- f^1 comprend une séquence complémentaire à la longueur correspondante de M3 et possède une extrémité 3' complémentaire à l'extrémité 3' de M3, et
- f^2 est complémentaire à une séquence de longueur correspondante dans F3 et possède une extrémité 5' qui commence au nucléotide complémentaire à l'extrémité 3' de F3;

G consiste en la séquence

5' g^1-g^2 3'

dans laquelle

- g^1 comprend une séquence identique à la longueur correspondante de M3 et possède une extrémité 3' correspondant à l'extrémité 3' de M3, et
- g^2 est identique à une séquence de longueur correspondante dans F4 et possède une extrémité 5' qui commence au nucléotide correspondant à l'extrémité 5' de F4:

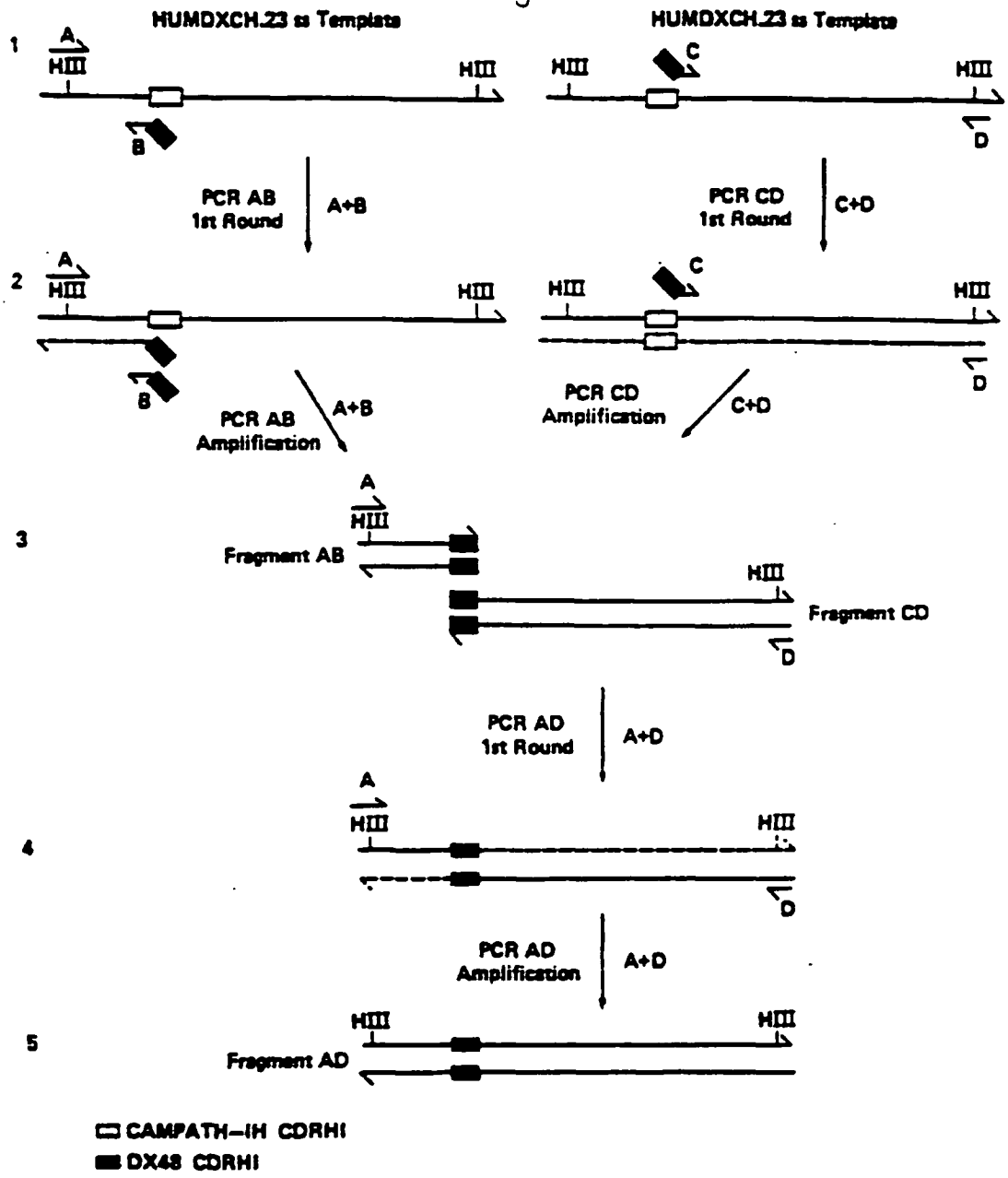
H

- comprend une séquence h^1 qui possède une extrémité 5' complémentaire à l'extrémité 3' de F4 et qui est complémentaire à une longueur correspondante de F4, et

- est orientée en sens 5' - 3' vers H3; et où les paires b^1 et c^1, d^1 et e^1, et f^1 et g^1 se chevauchent d'une longueur suffisante pour permettre l'annellation de leurs extrémités 5' l'une à l'autre dans des conditions permettant la réalisation d'une réaction PCR;

(iii) l'exécution, dans n'importe quel ordre souhaité, de réactions PCR avec des paires d'amorces A,B; C,D; E,F et G,H sur la matrice préparée au point (i) ci-dessus, pour produire des fragments d'ADN AB, CD, EF et GH; et
(iv) l'épissage des fragments obtenus au point (iii) ci-dessus pour obtenir l'ADN souhaité.

Fig. 1


HUMDXCH.23 as Template
HUMDXCH.23 as Template
1
A
HIII
HIII
B
HIII
C
HIII
D
PCR AB
1st Round
A+B
PCR CD
1st Round
C+D
2
A
HIII
HIII
B
HIII
C
HIII
D
PCR AB
Amplification
A+B
PCR CD
Amplification
C+D
3
A
HIII
Fragment AB
HIII
Fragment CD
D
PCR AD
1st Round
A+D
4
A
HIII
HIII
D
PCR AD
Amplification
A+D
5
HIII
HIII
Fragment AD
CAMPATH-1H CDRH1
DX48 CDRH1

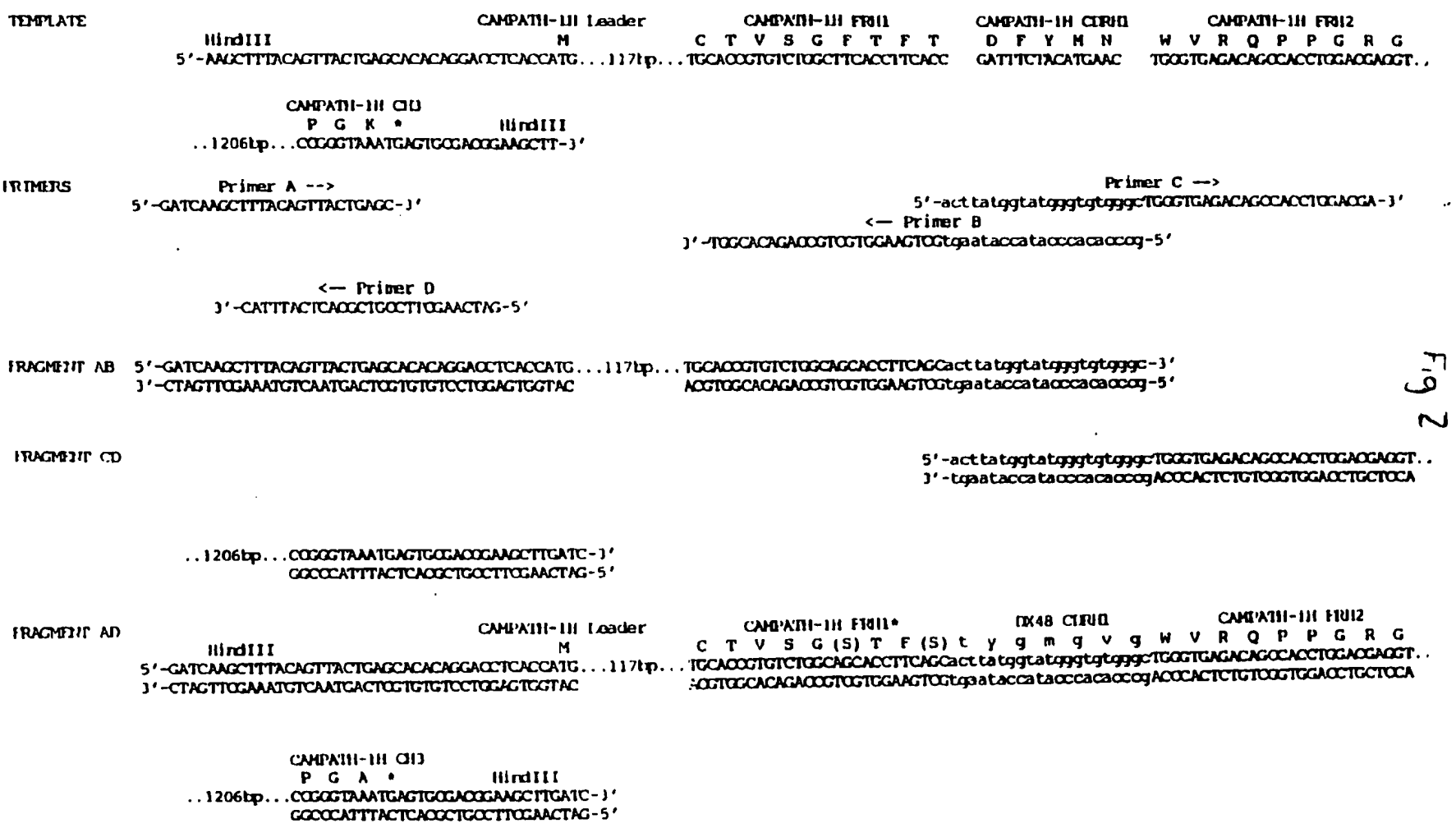


Fig 2

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Fig 3

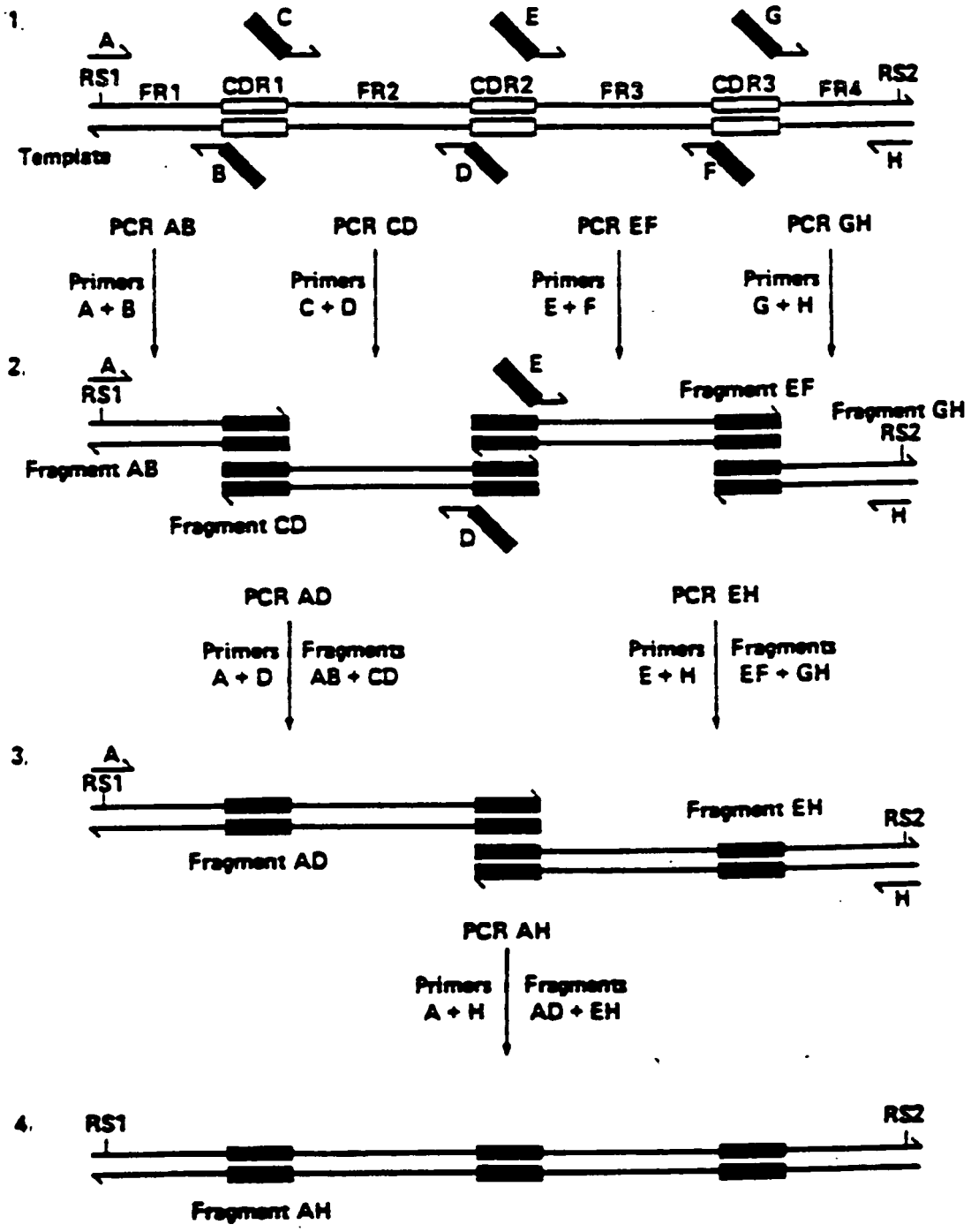
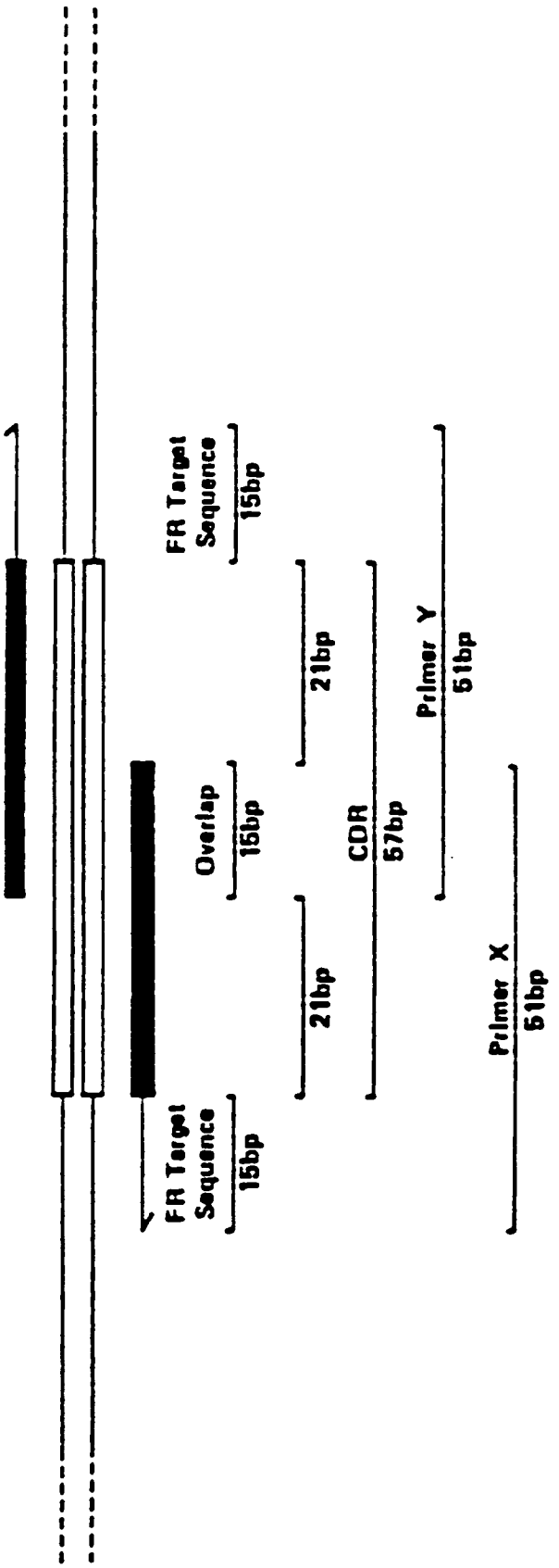

1.
A
C
E
G
RS1
FR1
CDR1
FR2
CDR2
FR3
CDR3
FR4
RS2
Template
B
D
F
H
PCR AB
PCR CD
PCR EF
PCR GH
Primers A + B
Primers C + D
Primers E + F
Primers G + H
2.
A
RS1
E
Fragment EF
Fragment GH
RS2
Fragment AB
Fragment CD
D
H
PCR AD
PCR EH
Primers A + D
Fragments AB + CD
Primers E + H
Fragments EF + GH
3.
A
RS1
Fragment EH
RS2
Fragment AD
H
PCR AH
Primers A + H
Fragments AD + EH
4.
RS1
RS2
Fragment AH

Fig 4


FR Target Sequence
15bp
21bp
Primer Y
51bp
Overlap
15bp
CDR
57bp
21bp
Primer X
51bp
FR Target Sequence
15bp